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Double Duty for CCL21 in Dendritic Cell Trafficking

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Mechanisms controlling leukocyte adhesion, propulsion and directional migration have not been fully integrated. In this issue of *Immunity*, Schumann et al. (2010) propose that DCs swarm to T cell zones using immobilized CCL21 for adhesive random migration and soluble CCL21 for steering.

The discovery by Metchnikoff more than a century ago that phagocytes migrate by crawling raised fundamental questions about the adhesive properties of the crawling surface and how they might facilitate movement. Adhesive migration is also referred to as haptic movement. The word "haptic," from the Greek "haptesthai" meaning "to touch," signifies the adhesion of a cell to a surface. Two principal types of haptic movement can be distinguished: haptokinesis, where "kinesis" is the Greek word for "motion or movement," and haptotaxis, where "taxis" is Greek for "order." Thus, haptokinesis is movement of a cell along a surface, but it is generally meant to convey random movement, as opposed to haptotaxis, which is meant to convey ordered or directional movement of a cell along a surface, where directional cues are provided by gradients of bound ligands recognized by receptors on the migrating cell. In vitro assays have also been used for identifying migration that is dependent on soluble chemoattractant ligands, which may be either chemokinetic in nature, if the concentration of chemoattractant is uniform and movement is nonvectorial, or chemotactic, if the concentration of chemoattractant is nonuniform and the cell moves in a directional manner defined by the gradient.

In the early 1990s, efforts to understand leukocyte transendothelial migration resulted in the multistep model of leukocyte trafficking, a synthesis of research in the areas of adhesion and directional migration (Butcher, 1991; Springer, 1994). Other models were developed in specialized sites such as the lymph node, where lymphocytes and dendritic cells (DCs) appear to migrate along tracks defined by fibroblastic reticular cells (FRCs) and conduits decorated by chemokines and adhesion molecules (Germain, 2006; Bajénoff et al., 2007). Chemoattractants were initially thought to be presented as soluble factors, but later evidence for tethering to surfaces was reported, and tethering mechanisms were identified, including genetically encoded transmembrane domains and binding to glycosaminoglycans (GAGs) (Handel et al., 2005).

Still, the precise mechanism of migration has been difficult to define, and certain details are not consistent or do not fit the models at all. In particular, diverse biophysical states of chemoattractants could be defined, including obligate soluble chemoattractants, obligate membrane-bound chemoattractants, and chemoattractants that could be either soluble or membrane bound, converted through cleavage by specific enzymes. Importantly, soluble chemoattractants have been found to induce adhesion-independent leukocyte migration (Lämmermann et al., 2008).

These complexities are particularly well-illustrated by the key lymph node chemokine receptor CCR7 and its ligands CCL19 and CCL21 (Förster et al., 1999). CCR7 is expressed on naive T cells, central memory T cells, and mature DCs. CCL21 and, to a much lesser extent, CCL19 are produced by FRCs. Why two chemokines exist that bind to the same homeostatic receptor with similar affinity has been a mystery. A potential key to understanding the specific roles of CCL19 and CCL21 is their striking difference in structure: CCL21 has an extended C terminus that mediates GAG binding, whereas CCL19 lacks this domain and as such is an obligate soluble chemokine. With these observations in hand, the field was poised for a new synthesis for understanding leukocyte migration, this time between kinetic and tactic mechanisms, as described for the CCR7 system in this issue of *Immunity* by Schumann et al. (2010). This elegant study, based on

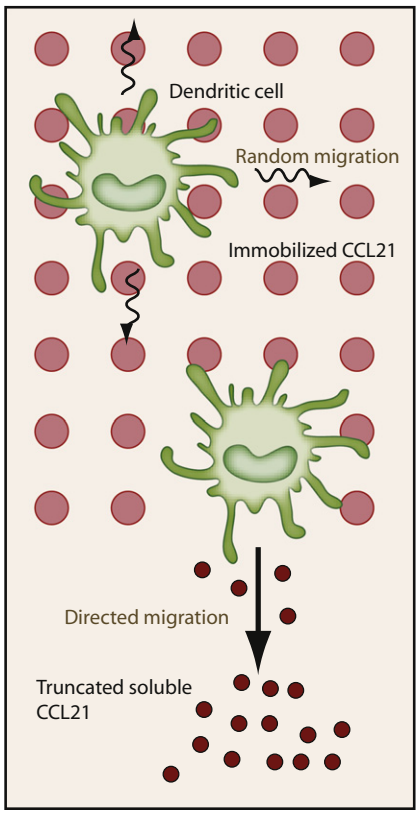

Figure 1. CCL21 Regulation of Mature Dendritic Cell Trafficking to T Cell Zones
In this model, proposed by Schumann et al., DCs undergo adhesive random migration (thin arrows) on fields of immobilized CCL21 (large red symbols) and ICAM-1 (not shown) that becomes directionally biased in response to concentration gradients of truncated soluble CCL21 (small red symbols), generated from the immobilized chemokine by the DC itself. Soluble CCL21 forms gradients and biases the direction of migration (thick arrow).

ex vivo and in vitro model systems of adhesion and migration, represents an important conceptual advance in the field.

The authors show stunning videos of mature DCs swarming across lymph node slices at specific physiologic entry points, concentrating specifically into T cells zone, where they stop. They show that bisecting the lymph node section and rotating one half of the section 180° relative to the other did not affect swarming, thus proving that directional cues in this system could not be haptic. So they searched for soluble factors. Because DC migration to the lymph node is known to require CCR7, the authors tested this receptor in their system and found that migration was dependent on CCR7 expression on the DC. Immature DCs and other CCR7 negative leukocytes were inactive in the assay. Moreover, DC migration on purified CCL21 in vitro appeared to be haptokinetic but was able to generate a soluble chemotactic factor that was also specific for CCR7. In vitro under agarose assays with molecularly tagged CCL21 tracked a proteolytic activity specific to the DC that was able to cleave the C terminus of CCL21 to release a soluble fragment that appeared to diffuse, form gradients, and provide a second nested chemotactic signal. Interestingly, the cleaved fragment corresponded to the GAG-binding domain of CCL21, the region that is lacking in CCL19, which mediates adhesion-independent chemotaxis. The authors found that CCL21 decorates the reticular network specifically in T cell zones in lymph node providing haptokinetic tracks for CCR7-positive DCs. Adhesion requires DC expression of β2 integrins, which are activated by immobilized but not soluble CCL21. Finally, the authors developed a 3D gel carbon fiber system to interrogate the importance of soluble chemokine in biasing the direction of migration. In this system, DC adhesion to the fiber required both ICAM-1 and CCL21 on the fiber and CCR7 and β2 integrins on the DC. Migration was nondirectional in the absence of soluble chemokine, but could be strongly directionally biased by the introduction of gradients of either soluble CCL19 or CCL21.

The principal significance of this paper is that it separates experimentally for the first time the adhesive and chemotactic function of a chemokine in the same system. The data support a model in which DC migration to T cell zones occurs on a reticular network decorated with immobilized CCL21 (Figure 1). As the cell interacts with the network, bound CCL21 triggers CCR7 to activate β2 integrins on the DC, thereby promoting cell adhesion to the network via ICAM-1-integrin binding and random migration. Then, a DC-specific protease cleaves CCL21 at the GAG-binding domain releasing a soluble short form of CCL21 that forms gradients able to directionally bias the cell and shape the migration path toward the T cell zone.

This two-step model has the virtue of being consistent with existing evidence in vivo for the distribution of total CCL21 and for adjustable leukocyte swarming within confined areas such as is found in lymph nodes. The authors have provided some in vivo support for it by directly demonstrating that a shortened form of CCL21 can be detected by immunoblot in extracts from mouse lymph node. Yet questions remain. Is this form soluble, and is it generated in large enough amounts relative to the putative soluble form released from FRCs before binding to GAGs? Moreover, does it form gradients, and is it located in the T cell zone to function as the authors propose? These are limitations to the interpretation and challenges for future experiments. In fact, difficulty with directly measuring chemokine gradients in vivo has been an undying source of angst and embarrassment for a field whose central dogma relies on the existence of such gradients.

Another challenge to the model is to explain why the proposed gradient of soluble CCL21 should develop in a manner favoring movement toward the T cell zone rather than away, or instead to produce homogeneous distributions of soluble CCL21. Also, it will be important to identify the DC protease able to cleave CCL21, to define the specific cleavage site, and to design in vivo experiments that might test the physiologic importance of this modification. In addition, it will be interesting to test whether the model is unique to the CCR7 system in lymph node, or whether it may apply generally to the entire chemokine system operating on tracks or at endothelial surfaces.

Most chemokines are similar in length and bind GAGs via multiple loop regions, not through terminal prosthetic domains such as CCL21 (Handel et al., 2005). Converting such chemokines from GAG-binding to soluble forms by proteolysis is probably not feasible. However, most chemokine receptors have multiple chemokine ligands, some of which could act preferentially on surfaces and others preferentially as soluble ligands (Murphy et al., 2000). This kind of information is simply not available for most of the chemokine system, so in this regard, the work of Schumann et al. (2010) should serve as a catalyst to fill this gap in knowledge. Efforts to extend the authors' findings to T lymphocytes, which also enter T cell zones in a CCR7-dependent manner, will be challenged by the requirement for an exit strategy, which does not apply to DCs. Most T cells transiting lymph node

are naive or central memory cells that continue to express CCR7 on the way out, presumably against the gradient the authors propose or by a different route altogether.

Perhaps most perplexing is why a chemokine should induce different functional responses depending on the state in which it is presented. Why do soluble CCL19 and CCL21 not activate β2 integrins on mature DCs, whereas immobilized CCL21 does? There is a substantial literature on the effects of GAG binding on chemokine quaternary structure, and the existence of homo- and heterodimers of chemokine receptors; perhaps this will be the next important synthesis in understanding of how leukocytes migrate.

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